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SINGLE UNIT ACTIVITY IN THE VISUAL CORTEX DURING CONDITIONING IN CATS

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Abstract. Multiple neuronal activity, recorded through chronically implanted electrodes, was analyzed. During acquisition and consolidation of alimentary conditioned reflexes to electrostimulation of the lateral geniculate body or optical tract, the patterns of neuronal activity in the visual cortex and sensory motor cortex became organized in a way different from that observed during pseudoconditioning. The majority of the neurons showed change in activity during both the isolated action of the stimuli and their simultaneous presentation. In stabilized conditioned reflexes, the activity of neurons in the sensory motor and visual cortex was interdependent. Neuronal indices of backward conditioned connections during activation of the reinforcing structures were analogous to the reactions of visual cortical neurons during the conditioned stimulus action and were manifested by an increase of discharge activity.

INTRODUCTION

The concept of backward temporary connections was primarily formulated by Pavlov (15) to explain mechanisms underlaying the acquisition of instrumental conditioned reflexes in dogs. For many years this idea has been experimentally developed in the laboratory of Asratyan by means of various behavioral and electrophysiological techniques. Special attention to backward connections has been also given by Beritov whose works were primarily focused an theoretical aspects of the problem.

It was shown (1–3, 13) that the pairing of two stimuli, which evoke their own reactions, leads to the formation of forward temporary connections, when the first stimulus is followed by the effect of the second. In addition backward temporary connections are formal when separate presentations of the second, reinforcing stimulus start to elicit the proper reflex of the first, signalling stimulus. On the basis of these data, Asratyan (1, 2) formulated the idea that during conditioning there occur two-way connections. With the accumulation of experimental data the two-way temporary connection grew from a phenomenological observation to a successful instrument for the study of the nervous mechanisms of conditioned activity. Should local transformations of electrographic parameters in the cortical projection of the conditioned stimulus be considered as the effect of backward influences, then, according to Asratyan (2), this influence plays a double physiological role. First, it participates in the occurrence of the local conditioned state, which is characterized by an increase in the excitability of the cortical projection of the conditioned stimulus. Second, it is manifested in the formation of the local conditioned reflex, representing a newly acquired form of interneuronal functional connections.

It is known (5, 6, 12, 14) that conditioning may be accompanied by both the enhancement and suppression of neuronal impulse responses in the cortical projection area of the conditioned stimulus. In addition, it may lead to involvement in the activity of new neurons. A question arises whether all of these listed forms of impulse transformations are related to the feedback influence or only some of them, and secondly, whether the similarity between conditioned and unconditioned neuronal responses is the manifestation of the feedback influences?

We showed previously (14) that in animals with stable food-seeking conditioned reflexes to the electrostimulation of the lateral geniculate body (LGB), the presentation of the reinforcing stimulus elicits a neuronal response at the projection of the signalling stimulus (visual cortex) analogous to that characterizing the conditioned reflex. Since in these experiments visual perception of the reinforcing stimulus, meat, was not excluded, it was possible that the pattern of neuronal activity of the visual cortex was related both by the modulating specific influences of the reinforcing stimulus, along the backward conditioned connections from the alimentary to the visual center, and by the direct influence of the reinforcing stimulus on visual reception. In order to explore the specific influences of the structures of the reinforcing stimulus on the structures of the conditioned stimulus along backward conditioned connections, in this study we used a method with milk administration into the oral cavity which excluded sight of the alimentary stimulus. Our main aim was to study neuronal indices of two-way conditioned connections on the model of conditioned reflexes with milk reinforcement, trained in cats to electrostimulation of the brain structure of the visual analyser.

METHODS

Experimental. Seven chronic cats were used. The alimentary conditioned reflex was elaborated to electrical stimulation of the LGB or the optical tract (OT). Recording of the neuronal activity in the visual and sensory motor cortical areas (representing the tongue and chewers) was done with a set of nichrome electrodes, each of 50 μm in diameter (5). Electrostimulation of the visual pathways consisted of stimuli 2 Hz in frequency and 5 s in duration. For 3 cats the CS-US interval of 2 s and for other 4 cats of 3 s was used. 2 cc of milk was administered directly into the oral cavity through in indwelling cannula. Each experimental session consisted of 15–20 trials.

Statistical. Neuronal activity of the visual and sensory motor cortical areas was measured before training, during pseudoconditioning, during consecutive stages of conditioning, and in special tests consisting in the presentation of the reinforcing stimulus applied before and after conditioning. The machonogram of licking was recorded simultaneously with the recording of neuronal activity. Recording of all electrical parameters was done on a general purpose electrophysiological apparatus UEFI-3, designed at the Central Design Office of USSR Academy of Sciences, and on magnetic tape of a Nihon Kohden tape-recorder. The analysis and statistical processing of multi-neuronal activity was done on Plurimat S–100 computer. The selection of spikes was carried out by considering the spike's amplitude and shape, and for this purpose the levels were set and the spike's shape was described by definite algorhythms (Fig. 1 *A, B*).

Post-stimulation histograms and cross-correlation histograms were made at the analysis epoch of 100 ms with 1 ms bin. Additionally, in order to obtain a clearer picture of spike distribution density, histograms were made at the given analysis epoch, post-stimulation density histograms (PSDH) and density histograms of cross-correlation (CCDH) (Fig. 1*C*) using the formula:

$$P_i = \frac{1/4\, A_{i-1} + 1/2\, A_i + 1/4\, A_{i+1}}{A_{max}}\ ;\ P_i \leqslant 1.$$

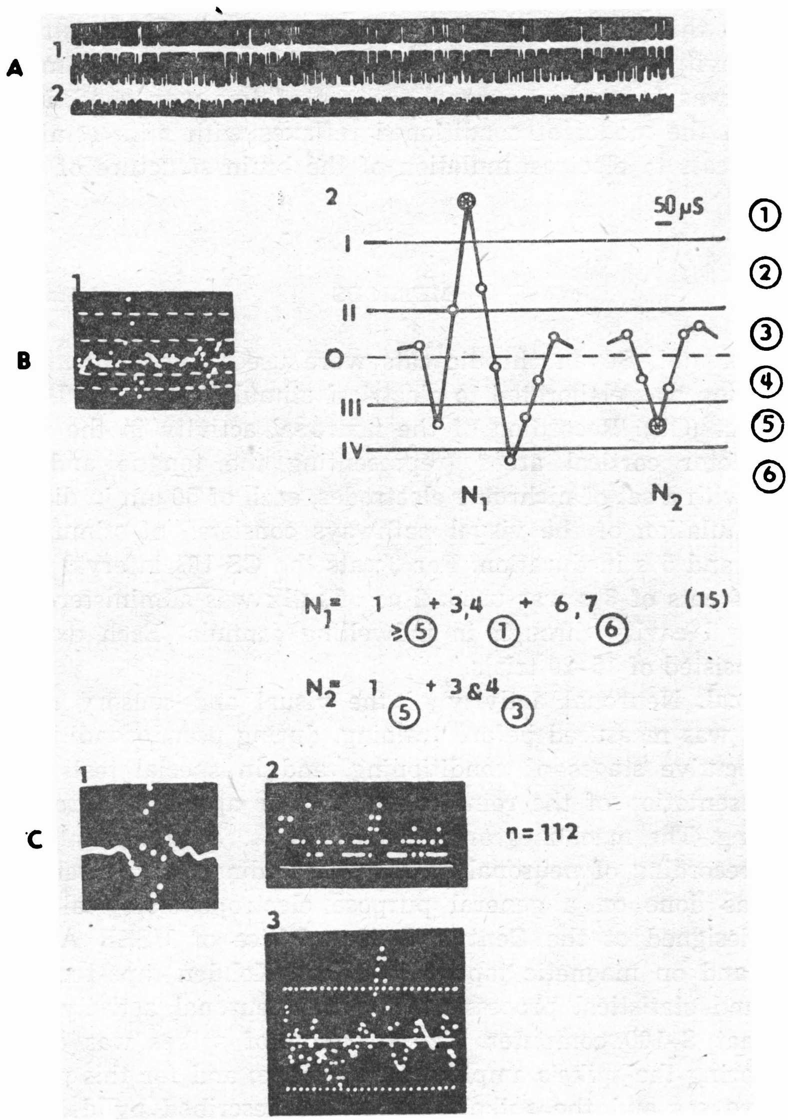

Fig. 1. Statistical analysis of chronically recorded multiple unit activity in the sensory motor (*A*, 1) and visual (*A*, 2) cortex of an unrestrained cat. Single action potentials with large and small amplitudes (*B*, 1); and diagramatic recording of the same action potentials (*B*, 2) illustrating the method of separating the large spike (71) from the discharges of its neighbors (N2), according to the algorithms shown below. Numbers in circles denote amplitude windows. For N1 point 1 occupied window 5; point 3 or 4, window 1; points 6 or 7 occupied window 6 and the next 15 points were excluded after the last point. For spike N2 point 1 occupied window 5, whereas points 3 and 4, window 3.

RESULTS

In five cats the neuronal activity was studied throughout the entire experiment and in two others only after stabilization of conditioned reflexes. The conditioned reflex was established after 30 to 50 pairings and manifested in the reaction of licking (Fig. 2).

Figure 3 shows three simultaneously recorded neurons from the visual cortex, (projection area of the conditioned stimulus) and sensorimotor cortex (representing the tongue and chewers) after stabilization of conditioned reflexes. In the impulse flows of both areas, neuronal responses similiar in shape were singled out and their reactions were studied on density post-stimulation histograms. It is seen that in the

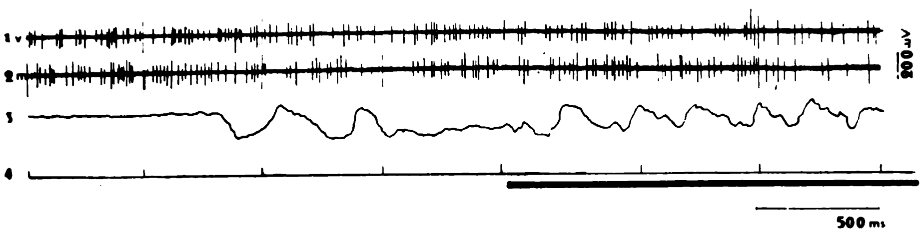

Fig. 2. Simultaneous recording of the electrophysiological and effectory indices of the conditioned milk reflex. 1, in the visual cortex; 2, unit activity in the sensory motor cortex; 3, machonogram of licking; 4, conditioned stimuli (vertical thin line) and reinforcement (heavy horizontal line). Trial 123.

visual cortex two neurons with different shapes and amplitudes (N1 and N3) responded to the conditioned stimulation with short-latency reactions. The first neuron of the motor cortex produced a cycle of excitatory responses with 40 Hz frequency, accompanied by periods of inhibition. Such forms of neuronal responses were typical of the motor cortical area and resulted from conditioning, because in the same animals they were absent before training. Thus, stable conditioned reflexes with milk reinforcement were characterized by a specific pattern of neuronal activity in the visual and sensory motor cortex.

We conducted special tests with activation of backward conditioned connections, which consisted of an isolated presentation of milk, and thus increased alimentary excitability. Figure 4 shows the original oscillograms of the stable conditioned reflex (*A*), the test with activation of the backward connection (*B*), and the intertrial licking after stabilization of conditioned reflexes (*C*). It turned out that in a number of cases, neurons of the visual cortex during isolated presentation of the reinforcing stimulus increased their frequency of discharges. The same was found for the intertrial licking movements after stabilization of conditioned reflexes (*C*), which was also considered as an electrophysiological manifestation of the backward conditioned connection.

In order to prove the functioning of the backward conditioned connection, we determined the statistical dependence of impulse neuronal responses of the sensory motor and visual cortical areas from which the cross-correlograms of neuronal activity pairs were drawn. Such analyses were performed for the background activity (30 pairs) and for the tests with activation of the backward conditioned connections (25 pairs) in the same animals before and after acquisition of the conditioned reflexes.

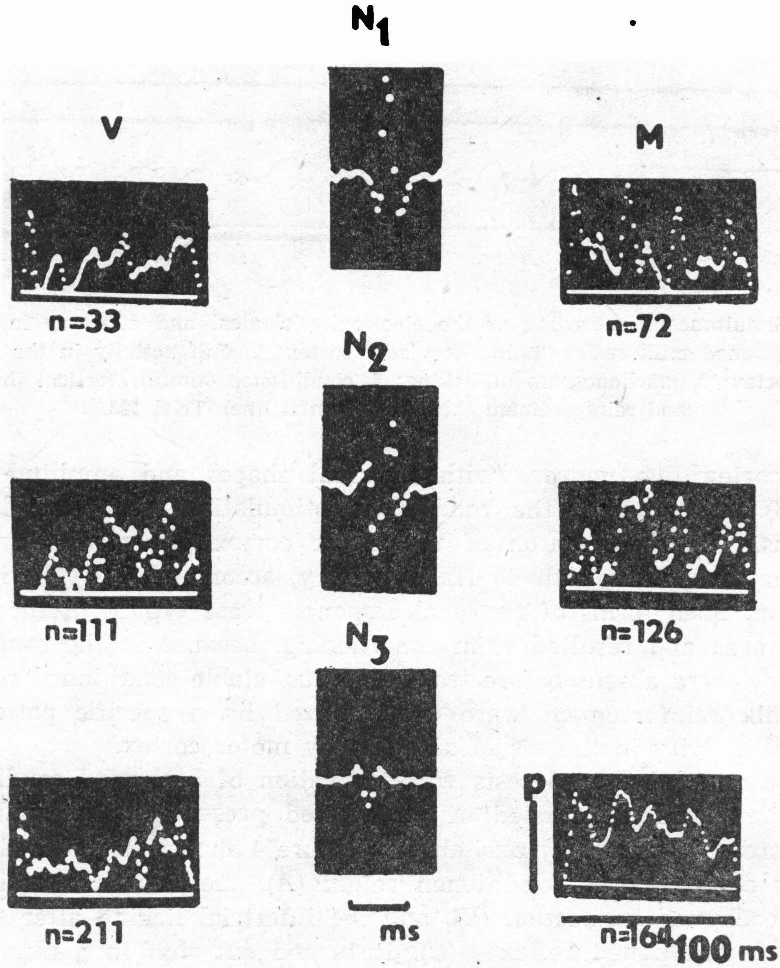

Fig. 3. **The shapes** (N1, N2, N3) **of the similar spikes singled out from multiunit activity and** their post-stimulus density histograms after conditioning. Visual cortex neurons (V), sensory motor (M).

Figure 5 shows density crosscorrelation histograms (CCDH) of cellular activity in the visual and sensory motor cortex. From the impulse row of the sensory motor cortex, a spike of maximal amplitude (M1) was singled out and intervals were studied, with which three different neurons in the visual cortex responded (V1; V2; V3). In constructing the

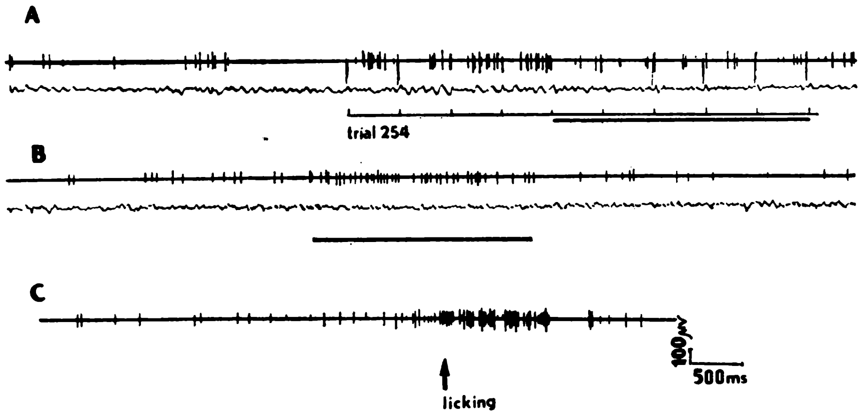

Fig. 4. The responses of visual units to the conditioned stimulus (*A*), to the isolated presentation of the milk test (*B*), and during intertrial licking (*C*) after stabilization of the conditioned reflex. The upper trace of each oscillogram shows visual unit activity; the middle trace represents EEG activity from the same point (both recordings were made by means of one electrode), the low trace marks the presentation of the conditioned stimulus and reinforcement. Increassing frequencies of spikes in each of three cases are shown.

CCDH, the impulses of neuron M1 (50) were presented to start the count, and impulses of neurons V1, V2, or V3, were counted for construction of corresponding CCDH shown in *B* and *C*. Some impulses of neuron M1 in the fixed analysis epoch were missing, but they were small in number because the averaged mean frequency of M1 discharges was 6–8/s. The symmetrical branch of CCDH was not investigated in the present study. It turned out that all three neurons of the visual cortex are connected differently with the motor neuron. On the CCDH it is evident that the maximal spike density of neuron V1 falls at 15 ms, that of neuron V2 at 30 ms, while that of V3 has an even distribution along the whole analysis epoch. The comparison of the CCDH obtained after acquisition of the conditioned reflex (C) and before conditioning (*B*) reveals that the neurons of the visual cortex V1 and V2 established clear excitatory connections with motor neurons, which was not observed in untrained animals.

In the analysis of 24 neuronal pairs during spontaneous activity before conditioning, 35% showed a statistical dependence of the visual neurons on the motor neurons (Fig. 6*A*, 1). Examination of the same 24 pairs after conditioning during intertrial periods indicated that 74% showed dependent relations, which was manifested predominantly in the form of excitatory connections (Fig. 6*B*, 1). A similar picture persisted in the special tests with activation of the backward conditioned

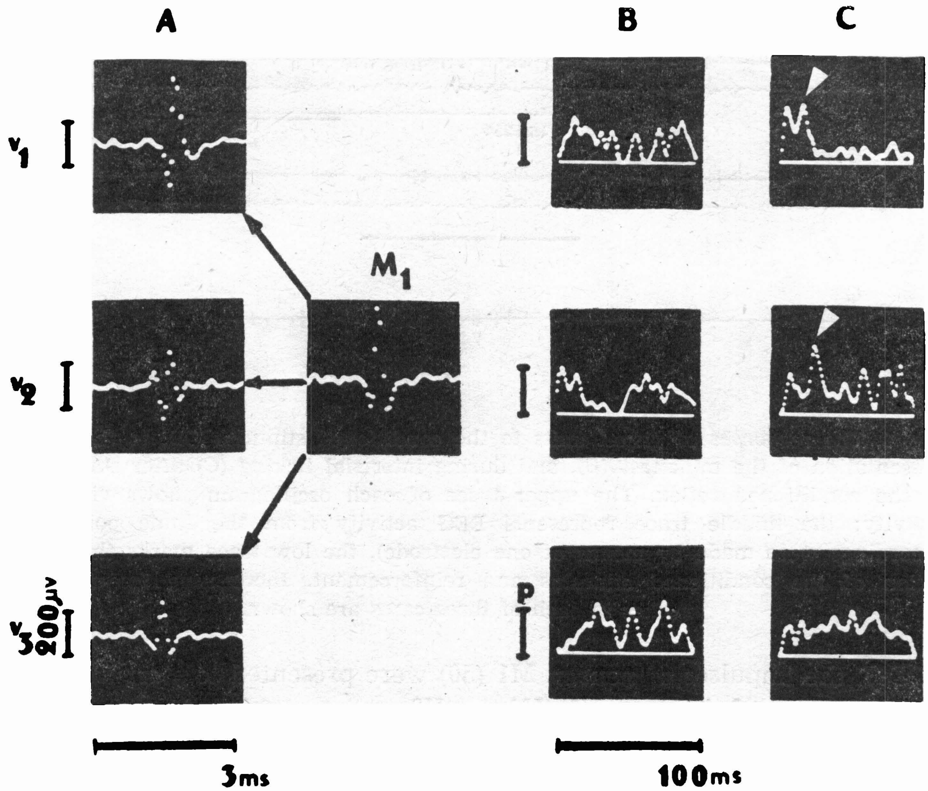

Fig. 5. Typical interactions between visual and sensory motor neurons before and after conditioning. *A*; V1, V2, and V3 represent three action potentials from each visual neuron to demonstrate their distinguishability. M1, a single action potential selected from sensory motor, multiple-unit activity. *B* and *C*: cross-corelation density histograms obtained from the selected neurons in the visual and sensory motor cortex of untrained (*B*) and trained (*C*) cats. The appearance the excitatory relation in the connection M1 — V1 and M1 — V2 can be seen in *C*.

connections when the reinforcing stimulus was presented alone. The analysis of 18 pairs showed that in 25% of the cases before (6*A*, 2) and 80% after conditioning (6*B*, 2) statistical dependence of visual neurons on the motor ones were found. Figure 7 shows the influences of different functional states on the pattern of relations between neurons of the visual and sensorimotor cortex. The increase of excitatory connections

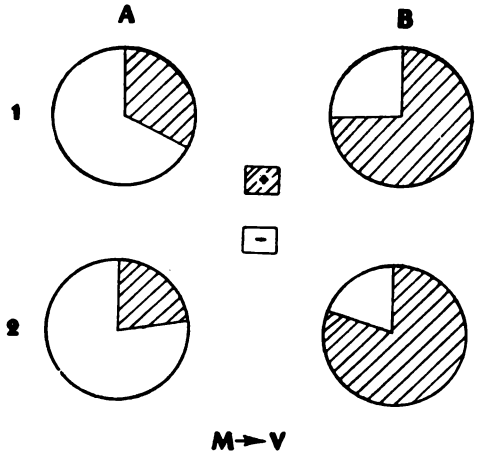

Fig. 6. Quantitative distribution of crossinterval histograms before (*A*) and after (*B*) conditioning. 1, sessions without testing milk presentation. 2, sessions with milk testing. The diagrams are drawn on the basis of data from 24 neuronal pairs concerning one type connections (from M to V).

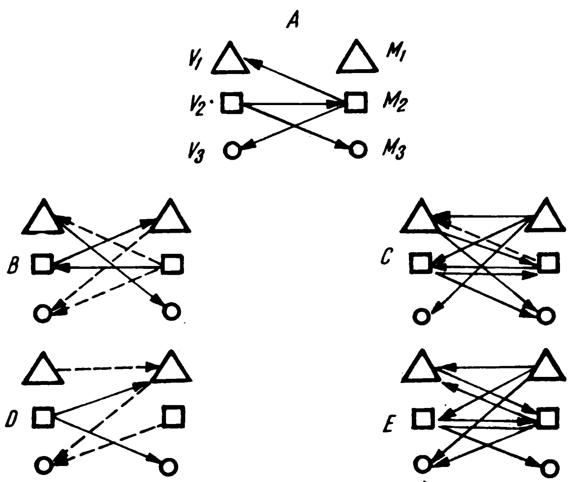

Fig. 7. The influence of different experimental situations on patterns of interneuronal connections drawn in the form diagrams. *A*, before conditioning; *B*, after pseudoconditioning; *C*, after conditioning; *D*, to the test presentations of milk before conditioning; *E*, to milk presentation after conditioning. The solid line indicates excitatory connection, the dotted line indicates inhibitory connections. Note the enhancement of connections between the visual (V1, V2, V3) and the sensorimotor neurons (M1, M2, M3) as well as the appearance of mutual interrelations after conditioning. Considerable increase of connections may be seen when the milk was presented after conditioning (*E*). All the diagrams are based on the analysis of 69 neuronal pairs.

after acquisition of the conditioned reflex and the appearance of mutual forms of interrelations did occur. The neuron V1 was supposed to form connections with the sensorimotor neurons (*C*), which did not exist before conditioning (*A*). The neuron M1, which was free of excitatory connections during pseudoconditioning (*B*) formed such connections with all visual neurons under study (*C*). The two neuronal group interrelations taking place during pseudoconditioning were less extensive and complex than those found during conditioning.

The difference of interneuronal organization during the milk presentation (*D*) was considerably more than without milk (*A*). As seen from Fig. 7, the visual-motor relations to milk testing were quite poor before conditioning (*D*) and extremely intensive after conditioning (*E*). The resemblance in the patterns of neuronal organization during conditioning in situations *C* and *E*, that is in sessions with and without the special milk testing, should be stressed.

DISCUSSION

We have shown the neuronal correlates of forward and backward conditioned connections in the process of acquisition and specialization of alimentary conditioned reflexes to electrostimulation of the visual analyzer. The elements of brain structures that perceive the reinforcing stimulus were activated along the forward connections. In the present model of conditioned reflexes these are the structures of alimentary and motor centers. Both the selective increase in activity of the neurons of the visual cortex and the statistical dependence between neurons of the sensory motor and visual cortex, were considered as the effect of afferentiation along the backward connections.

The existence of two-way conditioned connections supported the hypothesis put forward by Asratyan (2) who pointed out that "it is logic to assume that unconditioned reflexes, elicited by stimuli, paired in order to elaborate the conditioned reflex, are mutually reinforcing, that the process of reinforcement is also two-way by nature". In the cortical projections of each of the paired stimuli, proper interfocal functional connections were formed, which created active functional units. The latter were able to participate in the constellation of these foci, activity of which Asratyan called a "local conditioned reflex". It seems possible to consider all of the electrophysiological data, relating to changes in the discharge activity of neurons in the foci of paired stimuli, from the view of two-way reinforcement, recalling, however, that the power of these influences may be different.

The electrophysiological indices of the backward conditioned connection have been reported (2, 13, 14). Initially it has been reported that during performance of the intertrial classical motor conditioned reflex response, when only non-signalling repetitive cliks were presented, elicited in the auditory cortex evoked potentials, analogous to that evoked by the sporadic CS. It was suggested that only the backward conditioned connection between the sensorimotor and auditory cortical areas could provide a structural basis for this phenomenon. Our presented previous data (13, 14), also showed an increase in late evoked potential components, as well as neuronal correlates of backward conditioned connections in the form of activation of neuronal responses to the reinforcing stimulus in the visual cortex.

In the present study the influence of the reinforcing stimulus along the backward conditioned connections on the structures of the conditioned stimulus projection, were revealed both in special tests with activation of the backward conditioned connection and in the forming of a specific pattern in visual neuronal discharge activity in response to the conditioned stimulus. There are some data (8, 11) that in acquired reactions the changes of evoked potentials in the projections of the conditioned stimulus may reflect the influence along the backward conditioned connections. We assume, that the pattern of discharge neuronal activity in the visual cortex, shown in the response to the sporadic conditioned stimulus, is formed under the influence of spike activity, coming along the backward conditioned connections from the structures of the reinforcing stimulus. The methodological characteristics of our studies permit us to exclude the effect of alimentary stimuli on visual perception. We think that the pattern of neuronal activation in the visual cortex reflects the result of a functional neuronal interaction, formed exclusively by the acquisition of the conditioned reflex, in contrast to other studies, where animals always saw the food, which in itself might contribute to the formation of the conditioned neuronal reactions.

After conditioned reflex acquisition to the isolated presentation of the reinforcing stimulus, neurons of the visual cortex responded with reactions analogous to that found in the period of isolated activity of the conditioned stimulus. Thus we tested the functional state and readiness of neurons in the visual cortex, the cortical projection of the signal stimulus.

Our data suggest that after stabilization of conditioned reflex the pattern of neuronal responses in the visual cortex is a result of a new functional interaction between the neurons, rather than the effect of diffuse non-specific influences on the neurons, suggested by some authors (4, 16, 17). We probably saw a reflection of non-specific influences in neuronal responses of the visual cortex during pseudoconditioning, when electrostimulation of the visual analyzer and administration of milk were done at random. The organization of the neuronal activity in the visual cortex in these cases differed from that found during conditioning.

Our data also suggest that two-way conditioned connections function not only between the structures of the alimentary and visual analyzers, but also between the motor and visual cortical areas. The appearance of specific neuronal reactions of the visual cortex during intertrial licking after stabilization of the conditioned reflexes, and their absence in untrained animals support this suggestion. Moreover, we obtained an increase in dependent relations between the neurons of the sensory motor and visual areas after conditioning.

According to the literature (6, 9, 10), in the study of spike activity of three or more neurons on cross-interval histograms of each neuronal pair, the dependent relations are treated as the indices of functional interneuronal connections of the microsystem. Different types of influences (automated stimulation with clicks, or with pulse current, rhytmic auditory stimulation) cause plastic changes in neuronal connections. Each type of influence has its own distribution of connections (6). The formation of new interneuronal relations and the changing of the existing ones may have occurred when the activity of separate cells remained within the limits of their initial values. Thus the study of interneuronal relations on CCDH is a more sensitive parameter than the study of the changes in activity patterns of single cells.

The analysis of statistical dependence in the activity of simultaneously recorded cortical neurons is an indirect method of studying synaptic processes. The presence of such dependence of neuronal pair spike activity may indicate both direct synaptic connections between these neurons and the existence of an outside general source. To date there are several criteria for the estimation of crossinterval histograms. Correlations occurring on a cross-interval histogram within an interval of several milliseconds suggest direct synaptic connections between neurons (9, 10). It was shown (12) that the most common time interval, characterizing neuronal interaction of the visual and motor cortical areas is equal to 10 ms. Neuronal responses of the sensorimotor cortex to electrical stimulation of the visual cortex with a latency of 10 ms were recorded in alert rabbits in both intact and isolated cortical strips. The excitation caused by direct stimulation of the cortex seems to use approximately the same pathway for its proliferation, and along this pathway the interaction between the neurons in the state of spontaneous activity occurs.

We showed the dependence of neuronal activity in the visual cortex on the neurons of the sensory motor cortex with different latency, which may be considered as the manifestation of the functioning backward conditioned connection. Further studies of interneuronal connections, both of an intrafocal and interanalyzer character, will permit the delineation of the physiological composition of two-way conditioned connections.

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